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presented by the author

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THE SEAT OF FORMATIVE AND REGENERATIVE ENERGY.

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THE question as to the role of the cytoplasm, presents itself under two forms:

1. Is the cytoplasm a passive body, moving only as it is acted upon by external forces, or in response to influences emanating from the nucleus?

2. Or does it behave rather like an organized body, endowed with subtle powers of its own, and capable of automatic as well as responsive action?

There is a strong tendency at the present time to refer all kinetic changes in the cytoplasm to the agency of the nucleus, and to ascribe to the former the passive role of a nutritive substance. The kinetic phenomena of the egg during maturation and impregnation have already been considered in their bearing on this important question.[1] A number of decisive proofs of pure nuclear action were pointed out, and at the same time an attempt was made to support the opinion that the cytoplasm is capable of automatic as well as responsive action. The present paper is chiefly devoted to the consideration of phenomena displayed in the cytoplasm, and to the discussion of the question, whether the regenerative and formative power of the cell resides in the nucleus or in the cytoplasm, or in both taken as a highly complex physiological unit.

The Doctrine of Isotropy. — Pflüger's interesting experiments[2] with the amphibian egg to determine the influence of gravitation upon the direction of cleavage-planes, led him to conclude that the entire egg is "*isotropic.*" In other words, to quote from the

[1] Oökinesis. *Journ. Morph.*, I., 2, p. 227, December, 1887.

[2] Ueber den Einfluss der Schwerkraft auf die Theilung der Zellen. *Arch. f. d. ges. Physiologie,* XXXI., pp. 311–318, and XXXII., pp. 1–79. 1883.

author, "the fertilized egg possesses absolutely no essential relation to the later organization of the animal, no more than a snowflake stands in any essential relation to the size and form of the avalanche which under certain conditions develops from it. That the germ always gives rise to the same form, is due to the fact that it is always brought under the same *external* conditions." Immediately after the appearance of Pflüger's papers, it was pointed out by Agassiz and Whitman[3] that, "if gravitation were the sole guiding agency in cleavage, its effect ought to be *instantaneous*, and it should be possible to change the direction of a cleavage-plane already in progress." It was also shown that the *time* required to bring about a transposition of the third cleavage-plane, suggested *a corresponding internal transposition of the active protoplasmic matrix of the ovum, including of course the nuclei.* "If a body constituted like the ovum is restrained by artificial means from taking its normal position, a redistribution of material must immediately set in and continue until the equilibrium is restored. The active portion of the ovum, having a lower specific gravity than the passive nutritive elements, would eventually recover its normal position, and thus *the virtual axis of the ovum would inevitably right itself in spite of the inability of the ovum to rotate bodily.*" Later observations have fully verified these suggestions.

As now maintained by Born,[4] Hertwig,[5] Weismann,[6] Kölliker,[7] and others, the cytoplasm alone is isotropic, while the nucleus is the seat of the directive and form-giving power in development.

In this modified form, the doctrine of isotropy makes a much nearer approach to truth, but I believe that it is far from correct in its estimate of the functional importance of the cytoplasm.

The logical consequences of this view are clearly presented by Oscar Hertwig[5] (p. 306) in the following words: "*An die Kernsubstanz also sind die Kräfte gebunden, durch welche die*

[3] On the Development of some Pelagic Fish Eggs. *Proc. Amer. Acad. Arts & Sciences*, XX., p. 40, 1884.

[4] Biolog. Untersuch. *Arch. f. mik. Anat.*, XXIV., 1885.

[5] Das Problem der Befruchtung und der Isotropie des Eies. *Jenaische Zeitschrift*, XVIII., 1885.

[6] Die Continuität des Keimplasma's als Grundlage einer Theorie der Vererbung. 1885.

[7] Die Bedeutung der Zellenkerne für die Vorgänge der Vererbung. *Zeitschr. f. wiss. Zool.*, XLII., 1885.

organisation des Thieres bestimmt wird. . . . Es erscheint gleichgültig, ob bei der ersten Theilung der eine Kern sich mit der sogenannten animalen, der andere mit der vegetativen Dottersubstanz umhüllt oder ob beide Kerne sich in vegetative und animale Dottersubstanz in dieser oder jener Weise theilen. . . . Der Dotter ist nicht so organisirt, dass aus einer bestimmten Portion desselben ein bestimmtes Organ hervorgehen müsste." It is conceded (p. 304) that the cytoplasm may have a low grade of organization; but it is an organization that changes from moment to moment (p. 309), not a "*feste Organisation*" bearing fixed relations to the future organism.

The opposing view finds the differentiating and formative principle either in preformed elements of the cytoplasm ("*physiological molecules*" Lankester, "*polarized molecules*" Pflüger, "*idioplasma*" Nägeli), or in a definite organization of the cytoplasm itself (Van Beneden and others). "Though the substance of a cell," says Lankester[8] (p. 14) "may appear homogeneous under the most powerful microscope, excepting for the fine granular matter suspended in it, it is quite possible, indeed certain, that it may contain *already formed and individualized*, various kinds of physiological molecules." And again, "The development of one kind of cell from another kind is dependent on internal movements of the physiological molecules of the protoplasm of such cells."

Van Beneden[9] has made a thorough study of the structure of the egg of *Ascaris megalocephala* with a view to ascertaining if "les plans de symétrie de l'embryon ne se trouvent pas déjà préformés dans l'œuf lui-même et si l'un des traits les plus caractéristiques de l'organisation de l'espèce, la symétrie qui la distingue, ne se trouve pas déjà indiquée dans l'œuf. L'œuf d'un animal à symétrie bilatérale aurait-il, comme l'animal dont il provient et qu'il doit devenir, une extrémité antérieure, une extrémité postérieure, une face ventrale, une face dorsale, une droite et une gauche? les matériaux qui doivent servir à édifier la moitié droite du corps siègent-ils dans la moitié droite de l'œuf et la substance

[8] Notes on Embryology and Classification. London, 1877.

[9] Recherches sur la maturation de l'œuf et la Fécondation. *Arch. de Biologie*, IV., 2 & 3, 1883.

de la tête ne se trouve-t-elle pas concentrée en un point déterminé du corps ovulaire?"

Both views recognize the necessity of assuming that the course of ontogenetic development is in some way predetermined in the egg; but while one finds the *force motrice* in the nucleus, the other would locate it in the cytoplasm. The advocates of the former appeal to the so-called isotropy of the cytoplasm, to the conspicuous part played by nuclear bodies in fecundation and cleavage, to the incapacity for regeneration shown by enucleate fragments of infusoria, etc.; while the supporters of the latter insist on the constancy of premorphological relations (axial relations, relation of first cleavage-plane to the median plane of the future embryo), the remarkable structural features exhibited in some eggs, cleavage in planes not previously marked by karyokinetic division, etc. The truth appears to me to lie on both sides, the error consisting only in unduly exaggerating the relative importance of one or the other factor. Just now the weight of authority seems to be turning in favor of the first view, a result which must be attributed very largely to the influence of recent discoveries and theories respecting the nature of fecundation. The question is one of such fundamental importance, that it seems desirable to analyze closely the facts bearing on the subject. It is for this reason that I have dwelt more at length on the movements of the germinal vesicle and the pronuclei.

Especially important is the study of the structure of the egg, and the modifications which it undergoes during the period of maturation. One of the most important contributions in this direction is unquestionably Van Beneden's great work on Ascaris. No other biologist has yet gone so deeply and thoroughly into the subject, nor has any one discussed it with a keener appreciation of its theoretical importance. Such well-marked structural features as are claimed to exist in this egg are inconsistent with the idea that the cytoplasm is isotropic.

Polar Rings. — Among the more extraordinary examples of cytokinesis may be mentioned the polar rings in the egg of Clepsine, first described by Grube [10] (pp. 15–16), and recently more in detail by Robin [11] (pp. 97–105) and Whitman [12] (pp. 20–29, 39–41).

[10] Untersuchungen ueber die Entwicklung der Clepsinen. Königsberg, 1844.
[11] Mémoire sur le Développement embryogénique des Hirudinées. Paris, 1875.
[12] The Embryology of Clepsine. *Quart. Jour. Mic. Sci.*, July, 1878.

Although it is by no means certain that the hyaline protoplasm of these rings is not in part derived from the germinal vesicle, it is quite clear that the phenomena are very different from the polar phenomena attending the division of the first cleavage-nucleus. These remarkable exhibitions of polarity in the cytoplasm appear early in the pronuclear stage, and continue not only during the centripetal march of the pronuclei but even after the first cleavage-nucleus has entered upon its kinetic phases of division. *Thus we have two distinct series of polar phenomena in progress at the same time, one displaying itself in the cytoplasm, the other in the nucleus.* We cannot suppose that the cytokinetic series is dependent upon the karyokinetic series for two reasons: first, because the former begins earlier than the latter; and second, because such cytokinetic displays are unknown in other eggs. The second ground would also hold against referring them to pronuclear influences. Allowing that it may yet be possible to demonstrate that these movements originate in response to pronuclear influence, it would still be very difficult to believe that they are sustained throughout by the continuous action of the same agency. It would be altogether more probable, as a little reflection will show, that the movements once started are capable of maintaining themselves, independently of the inciting cause.*

Cytokinetic Phenomena. — The cytoplasm exhibits a great variety of changes and conditions, variously described as 'polar concentration,' 'radiating bands,' 'waves of contraction,' 'zonal constrictions,' 'automatic cortical layer,' 'amœboid movements,' 'phases of segregation,' 'astral radiations,' 'rhythmic contractility,' 'migratory movements,' 'crown of folds' (Faltenkranz), 'autonomic movements' (rotation, circulation, pulsation), etc.

While some of these phenomena might, with considerable reason, be claimed as purely cytokinetic, most of them are so intimately associated with karyokinetic activity that they must be explained, either as the direct result of the latter, or as the effect of impulses generated by the interaction of nucleus and

* The polar and parapolar circles described by Van Beneden (No. 9) in the egg of Ascaris, are not comparable with the polar rings of Clepsine. For the 'disque polaire' arises before, and disappears with, the penetration of the spermatozoon; the polar rings (Clepsine), on the contrary, appear after the penetration of the spermatozoon, and are not wholly dissipated until after the completion of the first cleavage.

cytoplasm. The hypothesis of reciprocal action is not incompatible with the opinion that the conditions of this action are furnished, in the first instance, if not continuously, by changes of a chemical or molecular nature, which arise quite independently, either in one factor alone, or in both. The source of the initiatory impulse would still be an open question. Our knowledge of the phenomena above designated is too incomplete to furnish a key to the solution of this problem. For the purpose we have in view, it will be sufficient, therefore, to refer to a few of the more important examples, in which each factor may be supposed to play a more or less important part, deferring the discussion of the main question until we come to consider phenomena of a more decisive nature.

Following the penetration of the spermatozoon into the ovum, various forms of contraction in the vitellus have been observed; and these are generally regarded as the effect of impulses generated by the spermatic element. The usual sequence of events certainly accords very well with this view, but there are one or two facts which should make us hesitate to accept it. In some aquatic animals, in which the sexual cells unite before ovipositing, the time of these contractions bears no constant relation to the time of union, but does bear such a relation to the time of contact of the egg with water, whether this contact be brought about artificially or in the natural course of events. Still another cogent reason for not ascribing these contractions to the *independent* action of the male pronucleus is found in the fact that similar, though more sluggish, movements may, in some well ascertained cases, be induced by placing *unfertilized* eggs in water. The most general of these movements is the flattening of the pole, and the gradual contraction of the whole vitelline sphere, resulting in the formation of a perivitelline space.

The Constriction Attending the Exit of the Polar Globules. — The flattening of the pole is attended, or followed, in some cases, with a very remarkable constriction, which, beginning in the equatorial zone, travels towards the animal pole, finishing up with a nipple-like protuberance, from which the first polar globule is expelled. The exit of the second polar globule is sometimes preceded by a similar but weaker constriction. This constriction has been observed (No. 12, p. 18) in the eggs of different species

of Clepsine; and the same, or a closely analogous constriction, has been described by Kupffer and Benecke[13] (p. 19) in the egg of Petromyzon, and by Ransom[14] (pp. 463, 464, 477, 479) in the egg of the Stickleback and some other fresh-water teleosts.

This constriction has been confounded by the last mentioned authors with yolk-contraction, and brought into connection with the formation of the perivitelline space ('breathing-chamber' of Ransom, 'Eiraum' of Kupffer and Benecke). This space probably results from contraction of the vitellus as well as from expansion of the egg membrane, but the constriction is a special act of the vitellus to expel the polar globule. The elimination of polar globules is thus a process involving co-operant actions of both factors; and if the part performed by the polar amphiaster is karyokinetic, the associated act of the vitellus may be characterized as cytokinetic. I have before referred to the centrifugal movement of the germinal vesicle as an instance of repellant action, and I regard this constriction as a part of the same action. *It is thus a phenomenon of maturation, not of impregnation.*

Ransom, as well as Kupffer and Benecke, explains the phenomenon as a result of the penetration of the spermatozoon, and hence has failed to distinguish it from other phenomena of a similar, though not identical, nature. Bearing this fact in mind, we are enabled to find in their descriptions — especially that of Ransom — an exact parallel of the special constriction which always accompanies the formation of polar globules in Clepsine. Ransom's account is extremely interesting, and has attracted so little attention from later embryologists, that it seems worth while to introduce a portion of it here. After stating that slow contractions begin from the first moment of entry of the spermatozoa, causing first a flattening of the germinal pole, and afterwards slight changes of outline due to 'travelling waves' at other parts of the surface, he proceeds as follows: —

"Gradually more vivid contractions commence, at various times after fecundation, according to the temperature. In warm weather they have been noted in six minutes, in cooler weather

[13] Der Vorgang der Befruchtung am Eie der Neunaugen. Königsberg, 1878.

[14] Observations on the Ovum of Osseous Fishes. *Proc. Roy. Soc. London*, VII., 1856.

in fifteen or twenty minutes after impregnation. They cause a flattening of one side of the yolk-ball, to see which it is often necessary to roll the egg over. *The flat surface gradually becomes a sulcus, giving a reniform outline to the yolk. It then extends all round, giving rise to a dumbbell shape. This sulcus, which may be termed equatorial, travels with considerable but variable rapidity towards the germinal pole, producing as it passes on, the flask form. The sulcus is lost by passing forwards to the germinal pole, not by relaxation. It is seen for a brief space affecting the thickness of the germinal disc only, to which it gives a nipple-like form, while the food-yolk is round. When effaced, the whole yolk-ball is globular and at rest, the germinal disc being no longer prominent. This series of forms recurs with more or less of regularity, and with some variations both of time and form, about fifteen or twenty times, each series being the result of a travelling wave*" (No. 14, pp. 463–464).

Had Ransom succeeded in connecting the formation of polar globules with the more regular and prominent 'wave,' which he has so vividly described, he would doubtless have seen the necessity of distinguishing this wave from the movements which follow it, precisely as Kupffer and Benecke distinguished in the egg of Petromyzon a 'zonal constriction' which invariably accompanies the appearance of the second polar globule. They did not succeed in tracing the origin of the first polar globule; but they have described a constriction (p. 15) around the germinal pole (Fig. 7, *l*), which appears immediately after the spermatozoa come into contact with the egg; and this, I would suggest, may have the same relation to the first polar globule that the 'zonal constriction' has to the second.

Polar Aggregation. — In the formation of the germinal disc of many pelagic fish ova, we meet with very remarkable cytoplasmic movements. In the fresh-laid egg, the germinal protoplasm forms a cortical layer of uniform thickness around the yolk. But this condition lasts only for a few seconds, during which the spermatozoon finds an entrance into the egg. This event is followed at once by a polar concentration of the peripheral layer of protoplasm, which results in the gradual formation of the germinal disc with its centrally placed pronuclei.

Is it in one or both of the pronuclear bodies that we are to look for the cause of the polar aggregation of protoplasm? Is

the protoplasm a passive mass, moved at the expense of nuclear energy alone, or has it motor energy of its own? In the latter case, are the conditions necessary to action supplied by the nuclei or by the protoplasm, or by both? Although we are yet a long way from a solution of these questions, it may be possible to show that the protoplasm is an active rather than a passive factor in the movements we are considering. Any view which represents the germinal protoplasm as a passive body, moving only as it is impelled by nuclear forces, appears to me irreconcilable with the following facts: —

1. In most meroblastic vertebrate ova (including those of many teleostei), the germinal disc is already formed before fecundation takes place. The *male* pronucleus cannot therefore be a necessary factor in the formation of this disc.

2. In many pelagic fish ova, where the disc forms after fecundation, the polar amphiaster is formed before polar concentration begins. The cause of concentration cannot therefore, in this case, be referred to the centrifugal movement of the germinal vesicle, nor to any changes which this body undergoes prior to the formation of the polar amphiaster.

If this conclusion holds equally in the first class of eggs, we are fully warranted in affirming that the germinal disc forms independently, not only of the male pronucleus, but also of the germinal vesicle and its derivatives, since in these eggs the disc is formed before fecundation and before the polar amphiaster divides.

The validity of these conclusions may be disputed by those who hold with Weismann (No. 6, pp. 90–122) that the two pronuclei are identical in their molecular structure, and that both act alike upon the protoplasm, but in proportion to their mass. It might be argued that a definite *quantity* of karyoplasm ('Keimplasm') is requisite in order to concentrate the protoplasm in the form of a polar disc. If the mass of the germinal vesicle, or of its pronuclear elements, be large enough, it would form a germinal disc without the aid of the male pronucleus; if it fall short of the requisite mass-measure, it would have to be reinforced by the male pronucleus before it could accomplish the work. It will be time to accept this view when it has been shown that there *are* such quantitative relations as the theory postulates. Such an explanation of the disc-formation would take no account

of the polarity of the egg, and would leave inexplicable the difference between telolecithal and controlecithal eggs. Besides, this view assumes that the pronuclei are homodynamous, a point which cannot be conceded, since male pronuclei do not behave towards one another as they do towards female pronuclei.

A special feature in the polarity of the fish egg, noticed by Kupffer[15] and by Hoffman[16] (p. 88), is the formation of a temporary discoidal thickening ('Gegenhügel,' Kupffer) at the vegetal pole. Here, then, is a disc-formation at the point farthest removed from the nuclear bodies, and this fact appears to be fatal to the above theory. We are reminded of the rings in the egg of Clepsine, and their concentration into two polar discs. It appears not improbable that the two sets of phenomena are similar in nature, and determined by like forces. In the fish egg the disc-formation is not preceded by a ring-formation; and the nearest approach to the ring-rays are the 'radiating bands' or 'beaded streams' described by Ryder[17] (p. 17).

Raffaele (Mitth. d. Zool. Station z. Neapel, VIII., 1, 1888) has recently described a very singular phenomenon in the egg of *Labrax*. The egg has a single large oil globule at the pole opposite the germinal disc. This oil globule is enveloped with protoplasm, which, on the side facing the germ, thickens up until it takes the form of a long club-shaped body. This body elongates in an axial direction, and the distal portion, which is gradually constricted off, eventually assumes a globular form and rests on the inner face of the germ. Ryder has noticed protoplasmic bodies in the egg of Gadus ('segmenting corpuscles') which, as Raffaele suggests, may have a similar mode of origin. It occurs to me that this body of protoplasm may correspond to Kupffer's 'Gegenhügel,' and that it is diverted from its usual peripheral track by the presence of the oil globule.

The Artificial Division of Infusoria. — The artificial division of infusoria has been resorted to as a means of deciding, experimentally, the question of the relative importance of the nucleus. M. Nussbaum[18] was the first to establish

[15] Die Entwickelung des Herings im Ei. *Jahresb. d. Comm. z. wiss. Untersuchung der deutschen Meere in Kiel*, IV.–VI., 1874–1876.

[16] Zur Ontogenie der Knochenfische. Amsterdam, 1881.

[17] The Embryography of Osseous Fishes. *Report of the Commissioner of Fish and Fisheries for* 1882.

[18] Die spontane und künstliche Theilung der Infusorien. *Arch. f. mik. Anat.*,

the general fact, that enucleate pieces of an infusorium are incapable of regenerating lost parts, while nucleate pieces soon regain the specific form. "*The nucleus is thus indispensable to the preservation of the formative energy of the cell.*" Gruber,[19] whose experiments were begun at about the same time as those of Nussbaum, has reached the same general result. But one of Gruber's experiments, which was at first supposed to show that regeneration is possible without the presence of a nucleus, brings out a fact of considerable importance. A *Stentor cœruleus* was selected, in which spontaneous fission had already begun, as indicated by the concentration of the rosary-formed nucleus into a simple oval form, and by the beginning of a new peristome at the middle of the body. A transverse section was made just in front of the new peristome, and so close upon the nucleus that it nearly all escaped from the cut surface of the posterior half of the Stentor. The anterior portion retained no part of the nucleus. The two parts were isolated, and on the following day each was found to have become a complete Stentor. As the plane of division was nearly the same as that which would have been followed if the process of spontaneous fission had not been interfered with, Gruber (No. 19, p. 13) finally concludes, contrary to his first interpretation, that this was not a case of regeneration, but of reproduction. The process of reproduction once initiated by nuclear action may go on, so he thinks, to completion without further assistance from the nucleus. He insists, however, that the first impulse to division is given by the nucleus, since in all cases where artificial division is executed *before* the process of spontaneous fission begins, enucleate parts are incapable of regeneration. While finding no reason to doubt the accuracy of Gruber's observations, I must contend that they do not warrant the conclusion so forcibly stated in the following words: "Auf rein empirischen Wege werden wir hier vor die unumstössliche Thatsache gestellt, dass *der Kern der wichtigste, dass er der arterhaltende Bestandtheil der Zelle ist*" (No. 19, p. 16). Allowing that regeneration is impossible in the absence of a nucleus, that is no proof that the nucleus is the sole seat of regenerative power, nor is it a proof that the nucleus is

XXVI., January, 1886, p. 485. Cf. also *Sitz-Ber. d. Niederrh. Ges. f. Natur- u. Heilkunde*, 1884, p. 262.

[19] Beiträge zur Kenntniss der Physiologie und Biologie der Protozoen. *Berichte d. Naturforsch. Ges. zu Freiburg*, I., H. 2, 1886. Also *Biolog. Centralbl.*, IV., p. 717, and V., p. 137.

a more important factor than the cytoplasm. There is not a single observation to prove what is so confidently asserted, that the nucleus gives the 'Anstoss' to division. This may be so and it may not. The observations prove, (1) that, in some of the higher Protozoa, *the whole process of reproduction by fission, with exception of the initiatory steps, may be accomplished independently of nuclear action; and* (2) *that the initiatory steps cannot take place if the nucleus and the cytoplasm are artificially separated.* The whole truth is well stated by Nussbaum (No. 18, p. 516): "*Kern und Protoplasma sind nur vereint lebensfähig:* beide sterben isolirt nach kurzerer oder längerer Zeit ab." It is clearly impossible therefore, by any such experiments as Gruber has carried out, to settle the question of the precise *locus* of the regenerative energy.

On general theoretical grounds, as well as by the fact that enucleate forms are found among the Protista, we are compelled to accept the generally received view, that the nucleus is secondary in origin. It may be true, as suggested by Gruber[20] (p. 151), that these so-called enucleate forms contain nuclear substance in solution, and that the first step in the phylogeny of the nucleus consisted in the formation of scattered granules, the coalescence of which would give rise to the single nucleus. But it is hardly necessary to add that we do not see how this (or any other) mode of explaining the origin of the nucleus as a *secondary* body can be brought into harmony with the idea that it embodies the whole regenerative energy of the cell.

Interesting and instructive as are these experiments in the artificial division of the higher Protozoa, they do not alone furnish a satisfactory basis for general conclusions. They require to be supplemented by similar experiments on the simpler forms of Protozoa, and by much more complete observations than have yet appeared on the normal processes of fission and coalescence exhibited in the Heliozoa. In proof of this we have only to refer to Gruber's own observations on *Actinophrys sol*[21] (pp. 63–67) and *Actinosphærium eichhornii*[22] (pp. 381–382). *Actinophrys*

[20] Ueber Kern und Kerntheilung bei den Protozoen. *Zeitschr. f. wiss. Zool.*, XL., 1, p. 121.

[21] Untersuchunge nueber einige Protozoen. *Zeitschr. f. wiss. Zool.*, XXXVIII., p. 45, 1883. Also *Zool. Anzeiger*, No. 118, 1882, p. 423.

[22] Ueber Kerntheilungsvorgänge bei einigen Protozoen. *Zeitschr. f. wiss. Zool.* XXXVIII., p. 372, 1883.

is capable of breaking up into parts without the concurrence of any visible changes in the nucleus. The enucleate individuals — if such they are entitled to be called — may agree perfectly with the nucleate individual in outward form and behavior. They live and grow, and coalesce not only with one another, but with the nucleate form. Whether they are capable of generating a nucleus or not was not ascertained. The multinucleate *Actinosphærium* breaks up in a similar manner without the intervention of the karyokinetic process; but here the individual parts generally contain one or more of the original nuclei. Two or more individuals may coalesce, but the coalescence extends only to the cytoplasm, the number of nuclei being the sum of those contained in the separate individuals before fusion. These remarkable facts appear to bear out Gruber's conclusion: "That the nucleus has no importance for those functions of the cell which do not stand in direct relation to reproduction; such as locomotion (pseudopod-formation), inception of food, excretion (pulsation of contractile vacuoles), and growth. Even on the external form it may be without influence" (No. 21, p. 66). Gruber (No. 19, p. 12) still maintains the accuracy of this view in every particular except that relating to the influence of the nucleus on the form of the cytoplasm. He now holds, in common with Weismann, Hertwig, Kölliker, Strasburger, and many other German biologists, that the form-creating and form-conserving principle is confined to the nucleus. How, when, or where the nucleus manifests its form-moulding power remains a mystery; but that it gives the first impulse to the regenerative act must be inferred — so the argument runs — from the fact that the violent separation of nucleus and cytoplasm destroys the regenerative power in all cases, except where the process of reproduction is begun before separation is executed. This is the focal point of the question with which we set out.

Aside from experiments in the artificial separation of nucleus and protoplasm, the principal arguments in support of this view are drawn from the phenomena of fecundation and cleavage, and are involved with certain theories of heredity which cannot be dealt with here. The observations of Gruber on *Actinophrys* and *Actinosphærium*, if the phenomena are not of a pathological nature, — and such an interpretation seems to be precluded, — should certainly make us hesitate to ascribe all the form-regu-

lating power of the cell to the nucleus. This reserve is rendered imperative by other facts yet to be mentioned, for one of which we are again indebted to Gruber[23] (p. 221). In summing up the results of his "Studies on Amœbæ," he states, "that two very closely related species of Amœba may have quite unlike-formed nuclei; while species differing widely in external form may have quite similar nuclei." Plainly this is the contrary of what might be expected if the formative power lay exclusively in the nucleus.

No Form-Correlation between Nucleus and Cytoplasm. — It may be put down as an indisputable fact that no *form-correlation* exists between nucleus and cytoplasm. Except during the process of division, the nucleus seldom departs from its typical spherical form. It divides and subdivides, ever repeating the same steps and ever returning to the same round or oval form. So far as can be seen, its influence upon the cytoplasm is equal in all directions; and hence it would seem that its formative power, if it have any, could only contribute to the maintenance of the spherical form of the cytoplasm. How different with the cell! It preserves the spherical form as rarely as the nucleus departs from it. Variation in form marks the beginning and the end of every important chapter in its history. While the nucleus repeats over and over again its little cycle of form-changes with mechanical regularity, the cell marches straight on from form to form, never returning, and never repeating, differentiating, developing, and adapting itself at every step to its environment and to the work it is destined to perform. From the egg onward through all the stages of histogenesis and form-evolution, we search in vain for a single intimation anywhere that either the form of the organism or the forms of the individual cells are moulded by direct nuclear influence. At the beginning of any ontogenetic series, when we get the most rapid and vivid displays of nuclear energy, we see that the environment of each cell is much more potent in determining its form than the nucleus. True, certain conditions of the environment may be said to be largely the result of nuclear activity; and to this extent the nucleus may be said to determine, indirectly, the form of the cell. But this is very different from saying that the nucleus has a direct controlling

[23] "Studien über Amoeben." *Zeitschr. f. wiss. Zool.*, XLI., p. 186, 1884.

power over the specific form of the cell, as claimed by Gruber, Weismann, and others. When the end of the ontogenetic series is reached, we find the reproductive power of the nucleus greatly diminished, and its influence over the form of the cell proportionally reduced. Indeed, in the majority of cases the form of the cell now appears to be maintained entirely independently of the nucleus, and whatever modification of form the latter exhibits appears to be the result of mechanical pressure. The form of the nucleus is now determined by that of the cell rather than the reverse.

If we study the more varied form-changes of the nucleus which occur in the life-cycle of one of the higher Protozoa, we are struck with the fact that the external form of the infusorium is, to all appearance, completely independent of what transpires in the nucleus. A single nucleus may divide a hundred times or more without the slightest effect on the form of the infusorium. The products of these many divisions may undergo various changes of form, and ultimately coalesce to form a single nucleus, and yet no change of form in the cytoplasm. The multinucleated form may break up into parts, some without, others with one or more nuclei, and the enucleate, uninucleate, and plurinucleate individuals all agree in presenting the same specific form. The nucleus may pass from the oval form to that of a long rosary; then, after a period of vegetative life on the part of the cytoplasm, return to the original oval form, and undergo the regular changes of division, the whole cycle of transformations coming to a conclusion without producing any discernible effect on the cytoplasm beyond that of simple fission. Each part carries with it the power to resume at once the form which characterized the original whole. The nucleus gives no evidence at any time of holding the formative power; but we have seen from Gruber's experiment on Stentor, above referred to, that the cytoplasm does exercise this power, and that *it does so even in the absence of a nucleus.*

Gruber does not attempt to deny this; but he thinks it necessary to assume that the power exhibited by the cytoplasm in the case mentioned, was communicated to it in the form of molecular motion, the original impulse being given by the nucleus. As we have seen, there is nothing in his experiments which makes such a conclusion necessary, and the burden of proof properly falls to him who makes the assumption.

One needs only reverse the case to see the illogical nature of the position. Let us suppose that it has been ascertained by numerous experiments that the nucleus of an infusorium is incapable of karyokinetic division when separated from the cytoplasm, except in those cases where the division is already in progress at the time of separation. Would there be anything either in the general rule or in these exceptional cases, from which to conclude that the performances of the nucleus are first set in motion by some impulse from the cytoplasm? Would the incapacity of the nucleus to divide when placed in abnormal conditions demonstrate its inability to divide autonomically under normal conditions? If artificial isolation were found insufficient to arrest a series of complex movements already begun in the nucleus, would the death of the nucleus, shortly after the completion of these movements, furnish any ground for denying that they were automatic? These questions appear to present the matter in a just light, and to carry with them their own answers. The point would hardly seem to deserve the attention given it, were it not for the great importance of the question under consideration, and the fact that we are dealing with the opinion of a high authority, — an opinion for which experimental evidence of a crucial nature is claimed, and an opinion fully indorsed by so eminent a thinker as Weismann.

Schneider's Experiment. — Schneider's [24] (p. 509) experiment with Thalassicolla, although not affording any decisive evidence as to the precise location of the formative power, is yet of some interest in this connection. The extracapsular or cortical protoplasm was removed, leaving the central capsule free. At the end of twelve hours the whole surface of the capsule showed delicate pseudopodial extensions, and soon after appeared a distinct extra-capsular layer ("matrix" of the pseudopodial rays), which gradually grew to normal thickness. The experiment was repeated three times in succession on the same individual, and each time with the same result. The extra-capsular envelope gave no evidence of sharing the regenerative power, but died shortly after isolation. This envelope, according to Brandt [25]

[24] Zur Kenntniss des Baues der Radiolarien. *Mül. Arch.* 1867, p. 509.

[25] Koloniebildende Radiolarien (Sphaerozoëen). *Fauna und Florad es Golfes von Neapel.* XIII. *Monographie*, 1885.

(p. 84) takes no share whatever in the production of spores; and thus it appears that the central capsule is the seat of the reproductive as well as the regenerative energy. During "vegetative life" the various functions (digestion, locomotion, sensation, etc.) all appear to be performed by the extra-capsular cytoplasm; but the whole functional activity, as supposed by Brandt, is under the *regulative* influence of the central capsule. In the Sphærozoa colonies the division of labor is carried still farther; for while the pseudopodial rays have their special functions, certain definite areas of the matrix ("Klumpen von Assimilationsplasma") provide for the digestion of the starch granules which are produced by symbiotic algæ; and different layers or zones are distinguishable even in the central capsule. That there should be such a complete separation of functions between the central core and the cortical layer of the same body of cytoplasm is no less instructive than it is remarkable. It is a capital illustration of the possibilities of organization and the physiological division of labor without any corresponding division into distinct morphological units.

Recent studies tend to show that the only important substance conveyed into the egg by the spermatozoon is that which takes the form of the male pronucleus. The unavoidable conclusion would appear to be that the pronuclei are the sole bearers of hereditary tendencies. This is unquestionably a point of cardinal importance, and it furnishes the strongest argument that has yet been advanced in favor of regarding the nucleus as the seat of the formative power. This side of the question could not be fairly dealt with within the limits of the present paper, as it would lead to a consideration of the whole problem of heredity. It is my purpose, however, to return to this subject at no very distant date.

The Idea of a Formative Power. — Let us now consider whether any rational basis can be found for the idea of a formative power as a resultant from, and an expression of, physiological unity. I am fully conscious that the subject is one of profound mystery, the solution of which appears to lie as far beyond our grasp to-day as at any time in the past. We draw nearer to the problem, but the effect is rather to enhance than to reduce its apparent magnitude. Every step in advance only brings us to a keener sense of the subtle and

incomprehensible nature of the force or forces contemplated. We see the effects only imperfectly, and are baffled in every attempt to understand the mode of action. For the present we must be content to search for the *direction* in which answers lie; and herein is found the chief value of theories.

The more important speculations on this subject have taken the form of theories of heredity. In most of these theories, at least those of recent date, we find a fundamental idea which must be accepted as true; namely, that the sexual cells reflect in some way in their chemico-physiological constitution all the typical structural features of the parent-organism. How all the hereditary tendencies can be contained in a single cell, and with such completeness that the developing organism repeats step for step the chief form-phases of a genealogical history stretching through countless myriads of generations, back from the present into the very dawn of life, and ultimately unfolds every detail of structure and feature of the parent-organism, is a mystery that transcends our understanding. The preformationists of last century took refuge in the celebrated inclusion (Einschachtelung, emboitement) theory, which made the real mystery unapproachable by hiding it behind an endless series of miracles. The triumph of the epigenesists brought with it the reclamation of the problem, but left us with the indefinable *vis essentialis* of Wolff, the *nisus formativus* of Blumenbach. In more recent times we have seen various metaphysical hypotheses of extra-organic agents or forces supplanted by physiological hypotheses which seek the cause of the phenomena in intra-organic forces. But the reaction which followed the fierce struggles with vitalism has left its impression on most of the theories now in vogue. Biological problems have been brought more and more under the influence of mechanical conceptions, which regard all phenomena from an objective standpoint. Science has vindicated this method, and as a *method* it is unassailable. It is no less indispensable to research in the organic than in the inorganic world; but the biologist is reminded at every turn that the method is not exhaustive, and from the nature of the case it never can be. The biologist does not hesitate to follow the shibboleth of "molecular motion" to its final goal, but he must beware of being blinded by any artifice of method to distinctions which lie

beyond it. He accepts the authority of the chemist and the physicist for the fact that the primary elements of the organism are identical with those found in inorganic matter, and with them repudiates the notion that life is "*a force having no connection with primary energy or motion.*" But no one disputes the fact that the living organism represents *special combinations* of matter and force, and displays phenomena which find no parallel in non-living matter. Hence it does not appear at all irrational to conclude that vital phenomena are the manifestations of special forces, resultants of course, and yet *quite unlike the elementary forces from which they are derived.*

If from the same elements by different chemical combinations, we get new substances, differing widely *inter se* in their chemico-physical constitution, and totally unlike their primary constituents, then why not new forces in the same way? The chemist and the physicist agree in referring the differences of substances to a dynamical cause, and their mechanical conceptions do not prevent, but compel them to ascribe a specific energy to each different atom and molecule. In spite of the tendency of physical thought to regard "all matter as one and all energy as one," chemistry and physics are built up on the assumption that chemical elements are unlike, and that in different modes of combination is given the basis for that infinite variety of substances with which we meet. If the primary energy is in each case called by the same name, "polarity," it is nevertheless understood that these polarities are as unlike as the elements which manifest them. It is just here that we see the foundation for those *qualitative* distinctions which, in the mind of the biologist, must ever overshadow in importance the physicist's factors of quantity and motion.

All physical explanations, no less than biological, lead ultimately to the conception of intrinsic forces. The chemist's unit, like the physicist's, is the embodiment of energy. From a comparatively few atom-energies an endless number of molecule-energies are built up; these aggregate in units of a higher order, some statical, others dynamical; and so on through what Nägeli calls *micellæ* and *micellar aggregates*, until we arrive at the living cell. In this ascending series each new aggregate represents a unit, the individuality of the parts being merged in that of the whole. The grounds for distinguishing various

organic, physiological, and biological units, are thus of the same general nature as those which compel us to discriminate between physical and chemical units. Of course these higher units combine both atomic and molecular structure; but they have superadded to, and including this, a structure as a whole, which is entirely ignored in the expression "molecular aggregates." As they result from the union, not of simple or complex molecules, but of complex molecular groups, their structure may be said to be at least as widely separated from the molecule as this is from the atom. The power which such a unit represents as a whole is not the same as the powers represented by its constituent elements when uncombined, nor is it the sum of these several powers. Derived from them, and yet wholly unlike them, as water is something totally unlike its chemical elements or any simple mechanical addition of these elements.

It is precisely this point which is so persistently ignored in all so-called physico-chemical theories of heredity. And yet all analysis and all observation leads to the conclusion, that molecular structure is not directly responsible for vital phenomena. In claiming that "physiological units" have something higher than molecular structure and power, I am not treading on ultra-scientific ground, but following the course already sanctioned by chemistry and physics, and the only one which can ever reconcile physico-chemical and biological conceptions.

Admit — what no one denies — that a molecule is totally unlike its constituent elements, that its energy is unlike that of its atoms, taken individually or collectively; and further, that simple molecules, without losing their structural integrity, may unite to form complex molecules, and we have only to carry the same process a few steps farther to reach those units whose integral structure is no longer adequately described as molecular. If analysis fails to discover a physiological bond which is capable of binding molecular aggregates into units of the vital order, its failure must be attributed to imperfection of methods, for observation bears constant and unvarying testimony to its existence. If analysis and observation combine to show that whatever force an organism expends is the correlate and equivalent of force taken into it, and if chemical and physical processes underlie all vital phenomena, it does not by any means

follow that there is any identity between vital activity and physico-chemical forces. The power which *causes* chemical elements to combine is not identical with the power which *results* from their combination; nor is the power which breaks down a chemical compound identical with the powers of its separate elements.

The views here enunciated are not contradicted by the long-established fact, that the laws which regulate the formation of chemical compounds are the same for both organic and inorganic bodies. The position taken does not affirm that organic compounds differ from inorganic either in material constituents, or in the forces which hold these constituents together. What we do affirm is this: We cannot stop with the most complex molecules revealed or revealable by chemical or physical research; we must pass from *organic* to *living*, *organized* matter, not by the supervention of new laws, but by ultra-chemico-physical, or chemico-organic combinations, which are absolutely beyond the highest possibilities of chemical analysis. Inability to define these higher modes of combination is no reason for doubting the testimony of all our senses to their existence. And why should we expect chemical research to bring any positive confirmation of their reality, when all chemical analysis presupposes conditions which are the absolute negation of vital conditions? Does self-stultification ever become more complete than in the assumption that vital forces and conditions are discoverable precisely there where they confessedly do not exist? Or is it rational to conclude that, because vital conditions have arisen from non-vital, the exclusive study of the latter will reveal the former? So long as the chemist's methods debar him from the study of physiological modes of aggregation will he be impotent to divine the links which connect molecular motion with sensibility, and just so long will "physiological chemistry" remain a delusive misnomer.

A complex aggregate of atoms, so bound together by mutual affinities as to represent a physical unit, possessing, as a whole, properties and powers derived from but unlike those of its constituent elements, and existing by virtue of, and only during the maintenance of, the chemical connexus of these elements, is a conception which may be carried straight forward up to the cell. The living cell may be regarded as a system of very com-

plex chemico-organic units, bound together by subtle chemico-physiological bonds, and displaying in their collective capacity functions and powers which are entirely foreign to them as individual and isolated elements, and which are therefore indissolubly identified with the physiological connexus or consensus.

Vague and unsatisfactory as such a view may appear, and as the best possible view must be from the limitations of our knowledge, it may yet contribute something towards a clearer conception of what we have called the formative power of the cell. It will be sufficiently clear now that we have not in mind a phantom-form which, like a mould, impresses its shape upon plastic material; but a power which represents the resultant of the consentient reactions of indwelling forces. Such a power declares itself in every living organism and in every developing germ.

The action of the formative power has often been likened to the architectural power displayed in crystallization; and if the essential distinctions are kept in view, such a comparison is justified by one or two very instructive analogies. If the physicist is not compelled to recognize a special crystallizing force, he is at least unable to deny that a crystalline aggregate reacts upon the parts in such a manner as to determine the *direction* of that marvellous "constructive power" with which the molecules are endowed. When we see a crystal reproduce its lost apex; or, as in the oft-cited experiment of Lavalle, an angle of an octohedral crystal spontaneously replaced by a surface, as the result of an artificially produced surface at the corresponding angle, we have no alternative but to infer a *physical correlation of parts*, under the influence of which the *direction* of forces is determined. So in the development of a germ, in the repair of injured parts, and in the regeneration of lost parts, the fact is irresistibly forced upon us, that *the organism as a whole controls the formative processes going on in each part.* The formative power then belongs only to the organism as a physiological whole; and it does not represent a sum or aggregate of atomic, molecular, or other forces, but results from special combinations of ultra-molecular units, and disappears as such the moment the physiological connexus is destroyed.

This idea may appear, at first sight, to stand in contradiction with the fact that parts of an organism, resulting from sponta-

neous or artificial division, possess the same formative power as did the undivided organism. But it must be remembered that most organisms do not admit of such division, and that in those that do admit of it, everything depends on how the division is made. The extra-capsular portion of a Radiolarian does not reproduce the central capsule, nor does the non-nucleated fragment of an infusorian regain its lost parts. Even here, then, it is not permissible to disregard the physiological correlation of parts, since both nuclear and cytoplasmic elements are indispensable to the preservation of the formative power. We still have to regard such organisms as physiological wholes, although the physiological connexus may be representable in aliquot parts.

The principle holds true of every organism, irrespective of whether the mass is divided into cells or not. The fact that physiological unity is not broken by cell-boundaries is confirmed in so many ways that it must be accepted as one of the fundamental truths of biology.